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(54) Apparatus and method for analyzing acquired scan data

(57) The invention refers to an apparatus and a method for analyzing and visualizing acquired physiological scan data. A display is used to visualize the acquired data and in addition to that the initial calibration setting can be done with sliders which are shown on the display. The apparatus is partitioned in a first subsystem a second subsystem. The second subsystem comprises a processing unit receiving the signals accumulated by the at least two detectors of the first subsystem. The seconds subsystem is used as well for calculating polar coordinates of the signals acquired by the first and second detectors. A unit for quantizing the angle component of the polar coordinates and adjusting the information capacity to the available display and a visualization unit for assigning color to the values measured are implemented in the second subsystem as well.

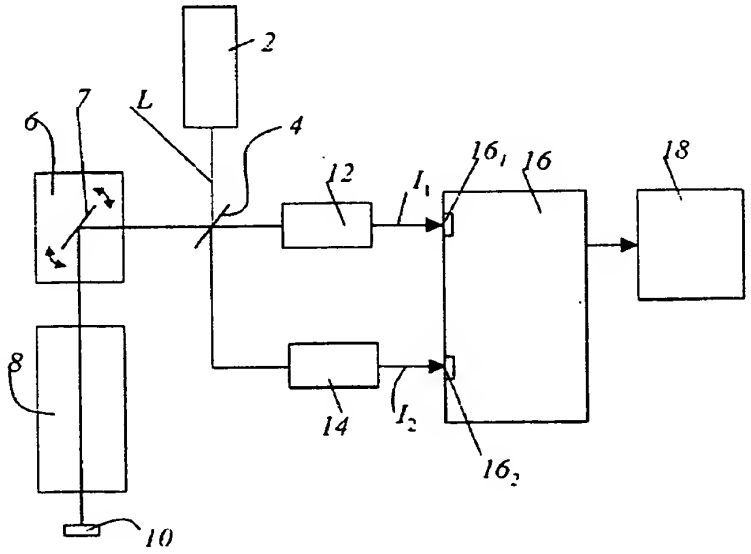

Fig. 1:

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physiological scan data and thereby improving the measurement results and the optimizing the visualization of the scan data and in addition to that allow a reduction in production costs.

[0008] This object is solved by an apparatus for analyzing and visualizing acquired physiological scan data. the apparatus comprises: a display; a first subsystem wherein the first subsystem comprises at least two detectors each responsive to a different wavelength; a second subsystem comprising a processing unit receiving the signals accumulated by the at least two detectors and calculating polar coordinates for the signals acquired by the first and second detectors; a unit for quantizing the angle component of the polar coordinates and adjusting the information capacity to the available display; and a visualization unit for assigning color to the values measured.

[0009] It is another object of the present invention to provide a method for analyzing and visualizing acquired physiological scan data. The method improves the measurement result and optimizes the visualization of the scan data.

[0010] This object is solved by a method which comprises the steps of:

- accumulating scan data for at least two different wavelengths each wavelength is accumulated with a different detector,
- transferring the accumulated data to a processing unit for calculating polar coordinates of the accumulated scan data
- quantizing the angle component of the polar coordinates and sending the data to a visualization unit,
- assigning color to angle components of the scan data for generating colored angle components, and
- displaying the colored angle components on a display.

[0011] It is a further object of the present invention to provide a scanning fluorescence microscope. The scanning fluorescence microscope analyzes and visualizes the acquired physiological scan data and thereby improves the measurement results and optimizes the visualization of the scan data and in addition to that allow a reduction in production costs.

[0012] The object is accomplished by a scanning fluorescence microscope with means for analyzing and visualizing acquired physiological scan data, comprising:

a display;
a first subsystem wherein the first subsystem comprises at least two detectors each responsive to a different wavelength;
a second subsystem comprising a processing unit receiving the signals accumulated by the at least two detectors and calculating polar coordinates for the signals acquired by the first and second detectors;
a unit for quantizing the angle component of the polar coordinates and adjusting the information capacity to the available display; and
a visualization unit for assigning color to the values measured.

[0013] It is yet another object of the present invention to provide a method for analyzing acquired scan data wherein the scan data are acquired with a scanning fluorescence microscope. The measurement results and the visualization of the measurement results should be optimized.

This object is accomplished by a method for analyzing and visualizing acquired physiological scan data comprising the steps of:

- providing a scanning fluorescence microscope having at least two detectors each of which acquire light which differs in wavelength,
- scanning a fine light beam generated by an illumination means for across a sample of interest,
- accumulating scan data for at least two different wavelengths each wavelength is accumulated with a different detector,
- transferring the accumulated data to a processing unit for calculating polar coordinates of the accumulated scan data
- quantizing the angle component of the polar coordinates and sending the data to a visualization unit,
- assigning color to angle components of the scan data for generating colored angle components, and
- displaying the colored angle components on a display.

In the preferred embodiment of the invention an apparatus for analyzing and visualizing acquired physiological scan data is disclosed. The configuration according to the present invention provides the advantage that in the context of the transformation, a separation is performed between the usable signal and the interference signal. This considerably simplifies the configuration of the apparatus, resulting in lower manufacturing costs. What is significant in this context is that after the transformation, only scalar variables are processed. The transformation maps the original two-dimen-

Equation 2 shows the ratio r as a function of a vector $\vec{\omega}$ that results from dividing first signal I_1 by second signal I_2.

$$r(\vec{I}) = \frac{I_1}{I_2} \qquad \text{(Equation 2)}$$

It is apparent that all the $r(\vec{I})=const$ in the I_1-I_2 diagram (see FIG. 3) define a straight line $g(r)$: $s_1 = s_2 r$. Each process that brings about a change in the ratio r must be associated with a change ΔI in the first and second signals equivalent to

$$\Delta\vec{I} = \begin{pmatrix} \Delta I_1 \\ \Delta I_2 \end{pmatrix} \qquad \text{(Equation 3)}$$

in which ΔI_1, and ΔI_2 represent the changes in the first and second signals I_1, I_2 respectively. This change ΔI can be separated into two directions: in a direction parallel to g, no change in the ratio can occur; in a direction perpendicular to the straight line g (corresponding to ∇r), a maximal change in the ratio occurs. Since this applies equally to all points, it is an obvious step to expand into polar coordinates as shown in Equation 4, in which R indicates the radius and φ the angle for representing the first and second signals I_1, and I_2 in polar coordinates:

$$\begin{pmatrix} R \\ \varphi \end{pmatrix} = \begin{pmatrix} \sqrt{I_1^2 + I_2^2} \\ \arctan(I_1 / I_2) \end{pmatrix} = \begin{pmatrix} R \\ \arctan(r) \end{pmatrix} \qquad \text{(Equation 4)}$$

As is evident from Equation 4, only angle φ contains the relevant information. In a scanning microscope (e.g. in a confocal microscope), the signals I_1 and I_2 deriving from first and second detectors 12 and 14 are necessarily discrete. In the simple case in which both variables are quantized in identical fashion in Q steps, the result is Q^2 distinguishable steps for the ratio signal. If identical probabilities are assigned to these steps, the number of bits shown in Equation 5 is needed in order to code the ratio.

$$B_{ratio} = ld(Q^2) \text{ bits} = 2ld(Q) \text{ bits} \qquad \text{(Equation 5)}$$

This corresponds to a doubling of the memory space as compared to the two acquired variables (first and second signals I_1 and I_2). Coding of the angleφ is determined by the number of vectors $\vec{r}$ that can be resolved on a circle of radius R for an angle φ from 0° to 90°. Assuming that R: $\phi < R \leq \sqrt{2Q}$ is constant, an upper limit of states can be indicated on the discrete space for each radius. This upper limit is defined by the length of the circular segment in Equation 6:

$$Q_R = \frac{\pi R}{2} \qquad \text{(Equation 6)}$$

Q_R is an upper bound for this value. This is an overestimate of states along oblique sections. This property is typical of discrete topologies. The maximum for this upper limit $Q_{R\,limit}$ is obtained, for $R = \sqrt{2Q}$, from Equation 7:

$$Q_{R,limit} = \frac{\pi\sqrt{2}q}{2}$$

Coding the states of this path (assuming an equal probability for all states) then requires a quantity B_R of bit states (Equation 8):

digital neural network.

Signal separation (physiological and non-physiological)

[0024] The separation of physiological signals from non-physiological interference can also be applied here. The formulas presented in the documentation of C. R. Bright et al.. Methods in Cell Biology, Vol. 30, pages 157 to 192, Academic Press Inc., for the calibration of physiological signals are all based on titration experiments. The in vitro calibration using Ca^{2+}ions is reproduced by Equation 12, the *pH* value by Equation 13, and *cAMP* by Equation 14:

$$[Ca^{2+}] = K\frac{r - r_{min}}{r_{max} - r} \quad \text{(Equation 12)}$$

$$[pH] \sim \log \frac{r - r_{min}}{r_{max} - r} \quad \text{(Equation 13)}$$

$$[cAMP] = K\left(\frac{r - r_{min}}{r_{max} - r}\right)^{1/n} \quad \text{(Equation 14)}$$

in which r_{min} is the measured ratio without calcium ions and r_{max} the measured ratio with the maximum concentration of calcium ions. All the underlying processes are delimited by one r_{min} and one r_{max}. When measuring r_{min} and r_{max} with fluorescent dyes, consideration must also be given to unbound and saturation states, meaning that in one case a maximum first signal I_1 is received and in the other case a maximum second signal I_2. These values must necessarily be measured with the same detectors, and are absolute operating limits. In I_1-I_2 diagram 24 (cf. FIG. 3), this information can be used to define a working region (in this case ignoring any background fluorescence, which essentially causes a displacement of the origin), within which a physiological signal must remain. White region 20 marked in I_1-I_2 diagram 24 is referred to as the "permitted region." Region 22 marked with crosshatching is of no physiological origin, and is not utilized for measurement. Any measurement point that is received in the hatched region is unequivocally a measurement error. This can be used to validate the measurement results. One possibility is to assign a specific color to not useable measurement results (for example by coloring points in the image red). In addition, this delimitation could also be used to reduce memory even further (although this is not discussed further here). The oblique operating boundaries can advantageously be verified in the transformation space that is described (boundary angles), and saturation intensities can be verified in the non-transformed space. Here again, an implementation in analog electronics, digital electronics, or software are possible. The segmented signal can be visualized directly; in an image, for example, all physiological components can be coded green, and all invalid components red.

Calibration and validation of physiological phenomena:

[0025] White region 20 depicted in FIG. 3 is the aforementioned working region, which is of high quality when it possesses the greatest possible opening angle and the largest possible surface area in I_1-I_2 diagram 24. This quality criterion can easily be verified, and is particularly favorable for use in an automated system adjustment method. One example is definition of the external corner points while at the same time displaying the I_1-I_2 plane in a derived form. FIG. 4 shows a configuration that is suitable for visualizing the aforementioned criteria. First and second signals I_1 and I_2 are delivered to control and processing unit 16. Also provided upstream from control and processing unit 16 are a first and a second branch 30 and 32, which convey first and second signals I_1 and I_2 to first and second comparison elements 34 and 36, respectively. Angle coordinate φ of the polar coordinate of the particular measurement point is similarly conveyed to a third comparison element 38, which defines the lower limits of angle coordinate φ. Angle coordinate φ is additionally conveyed to a fourth comparison element 40, which defines the upper limits of angle coordinate φ. Each of the comparison elements possesses an output 34a, 36b, 38a, and 40a, through which the signals are sent to a validation unit 42. The corresponding signals are sent from validation unit 42 out to display 18, and presented to the user in visual form.

[0026] FIG. 5 shows a schematic depiction of the invention which illustrates the correlation among the individual sub processes. The data to be processed are sent to a first section 50. The data derive, for example, from a corresponding receiving unit 50a. As described above, the data possess a particular coding of the amplitudes of signal pairs. This is a transformation of the amplitude vectors followed by quantization (see description of FIG. 2). The result of the transformation is to separate the usable signal and interference signal. This simplifies the system design and results in lower

70. The color information is adapted to the expected measured values. The algorithms implemented in visualization unit 75 are not specified further here. All algorithms profit from the improved signal dynamics that can be achieved with control and processing unit 16. For image data, accumulation or filters (for "attractiveness" purposes) could be integrated. For volumetric data (for example in confocal microscopy), there could be a volume renderer.

[0033] FIG. 7 shows one possible presentation of the data on display 18 after segmentation. The segmentation model of itself, however, can be visualized by way of a geometric analogy and presented to the user. This can be done with a presentation as in FIG. 7, similar to a cytofluorogram. First signal I_1 is plotted on the ordinate, and second signal I_2 on the abscissa. The individual measurement points are depicted as a cloud between the coordinate axes. What is depicted is the I_1-I_2 space as in FIG. 3, together with frequency information indicating how often each particular combination of the first and second signals (I_1 und I_2) occurs in an image. This space can also be used to visualize the segmentation polygon.

[0034] FIG. 8 shows an exemplary embodiment for adjusting a segmentation polygon 80 on display 18. The simplest form of calibration has an input mask for r_{min}, r_{max}, I_{min}, I_{max}. The gain and zero point are set by way of the system (generally using a panel box or slider, which is depicted correspondingly on the screen). A corner 82 of segmentation polygon 80 located opposite the coordinate origin can be adjusted using a first slider 84 on the abscissa and a second slider 86 on the ordinate. A third slider 88 on the abscissa and a fourth slider 87 on the ordinate make it possible to adjust the upper limit $I_{1\text{-}max}$ of first signal I_1 and the upper limit $I_{2\text{-}max}$ of second signal I_2. All the values can be read off directly from a diagram of this kind. At the same time, it is possible in this fashion to determine the quality of the measurement, which depends on the surface area of polygon 80. In addition, as in the case of a cytofluorogram, the image information can also be presented as a point cloud and the outliers-those points lying outside polygon 80-are directly visible. This information can be used accordingly for calibration. This is done by displaying the image and the diagram (FIG. 8) side by side on display 18. The average intensities in this polygon 80 yield the sharp corner of the polygon close to the origin of the coordinate system. The cell of interest could also be delimited by a polygon 80 in the image. The averaged intensities inside this polygon correspond to a second point.

[0035] FIGS. 9a through 9h are a graphic depiction of the calibration procedure for determining the polygon. FIG. 9a shows the first step in calibration. In diagram 90, a background region H is defined. A region of interest F represents, for example, the cell being examined or the cell of interest. The polygon can then be constructed sequentially using these two regions (or points) H and F.

[0036] As shown in FIG. 9b, images are continuously received and the user adjusts the zero points of first and second detectors 12 and 14 until background region H is located in the vicinity of origin 92. Background region H (bounded by a dashed line in FIG. 9b) then migrates toward the origin as indicated by arrow 91.

[0037] The ion of interest in the specimen is bound by chemical intervention. As FIG. 9c shows, region of interest F migrates as indicated by an arrow 93. Region of interest F achieves an increased intensity in diagram 90. The chemical intervention is done for example by pouring a solution of the ion of interest onto the sample.

[0038] In FIG. 9d, the gain of first detector 12 is adjusted so as to reach almost the end of the detector sensitivity/resolution. Region of interest F moves in the direction of an arrow 94 away from the abscissa of diagram 90.

[0039] As is evident from FIG. 9e, from the positions of the background region H and region of interest F it is already possible to define a first and a second line 95 and 96 of the segmentation polygon. First line 95 connects the background region H and the region of interest F, and second line 96 passes through region of interest F and runs parallel to the abscissa of diagram 90.

[0040] In FIG. 9f the ion of interest is now released by intervention. The region of interest F migrates in the direction of an arrow 97 away from the intersection of first and second lines 95 and 96. The intersection is identified by a dotted box.

[0041] In FIG. 9g, the gain of second detector 14 is adjusted so as to reach almost the end of the detector sensitivity/resolution. The region of Interest F migrates in the direction of an arrow 98 parallel to the abscissa of diagram 90.

[0042] As is evident from FIG. 9h, the final position of region of interest F allows a third and fourth line 99 and 100 to be defined. Third line 99 connects the background region H and region of interest F. The fourth line passes through region of interest F and is parallel to the ordinate of diagram 90. This algorithm maximizes the surface area of the resulting polygon, can serve as an explanatory component, and encompasses almost all the calibration parameters. These can subsequently be saved and reused when necessary (similar cells, similar environment). The steps described in FIGS. 9b, 9d, 9e, 9g, and 9h can be performed automatically. Each step in itself is relatively simple. The steps described in FIGS. 9d and 9g can be performed either by directly modifying the gain on the panel box or also by dragging region of interest F on display 18.

[0043] Although specific embodiments have been illustrated and described herein, it will be appreciated by those skilled in the art that any arrangement which is calculated to achieve the same purpose may be substituted for the specific embodiment shown. This application is intended to cover any adaptations or variations of the present invention. Therefore, it is manifestly intended that this invention be limited only by the following claims.

87	Fourth slider
88	Third slider
90	Diagram
91	Arrow
92	Origin
93	Arrow
94	Arrow
95	First line
96	Second line
97	Arrow
98	Arrow
99	Third line
100	Fourth line
I_1	First signal
I_2	Second signal
$I_{1\text{-max}}$	Upper limit of first signal
$I_{2\text{-max}}$	Upper limit of second signal
F	region of interest
H	background region
L	Light beam
r_{min}	Calibration parameter
r_{max}	Calibration parameter

Claims

1. Apparatus for analyzing and visualizing acquired physiological scan data, comprising:

   a display (18);
   a first subsystem (60) wherein the first subsystem (60) comprises at least two detectors (12, 14) each responsive to a different wavelength;
   a second subsystem (61) comprising a processing unit (16) receiving the signals accumulated by the at least two detectors (12 and 14) and calculating polar coordinates for the signals acquired by the first and second detectors (12 and 14);
   a unit for quantizing (70) the angle component of the polar coordinates and adjusting the information capacity to the available display (18); and
   a visualization unit (75) for assigning color to the values measured.

2. Apparatus as defined in claim 1 wherein the second subsystem (61) comprises a segmenter (72) which has a plurality of input ports for receiving the detector signals and angle component, and inputs for calibration parameters consisting essentially of minimum angle, the maximum angle, maximum and minimum intensities from the detectors, and a visualization unit (75) for assigning one color to proper measurement values and an other color to improper measurement values.

3. Apparatus as defined in claim 1 whereIn a storage unit (71) is provided for storing the quantized angle component of the polar coordinates.

4. Apparatus as defined in claim 2 wherein the processing unit (16) has a second output (68) for the radius component of the polar coordinate which is sent to a second quantizer (69) and the output of the second quantizer (69) is sent to the visualization unit (75).

5. Apparatus as defined in any one of the claims 1 to 4 wherein a scanning fluorescence microscope has the apparatus implemented.

6. Method for analyzing and visualizing acquired physiological scan data comprises the steps of:

   - accumulating scan data for at least two different wavelengths each wavelength is accumulated with a different detector (12 and 14),

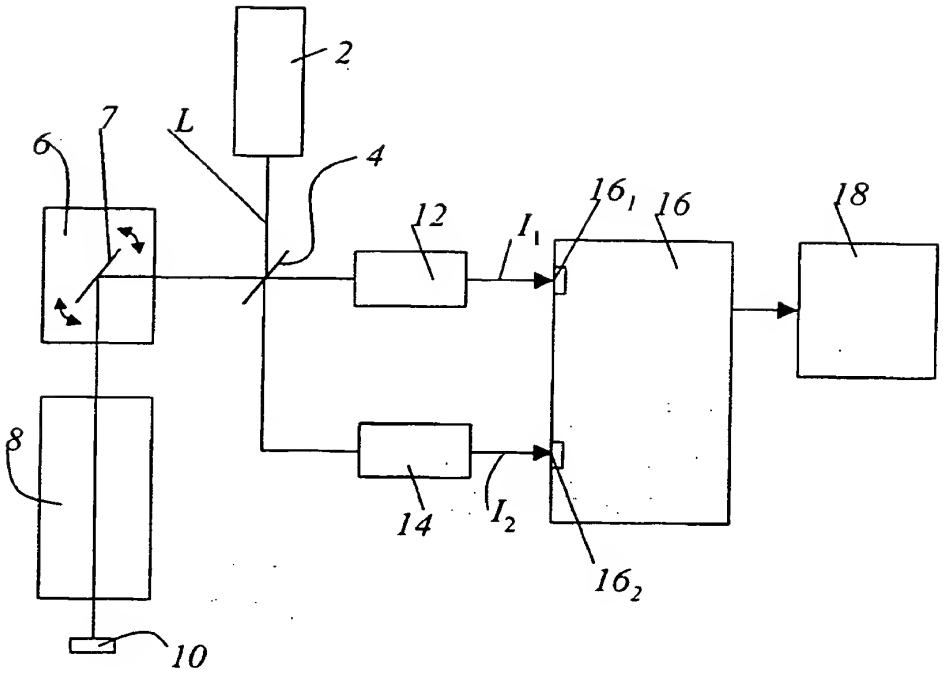

Fig. 1:

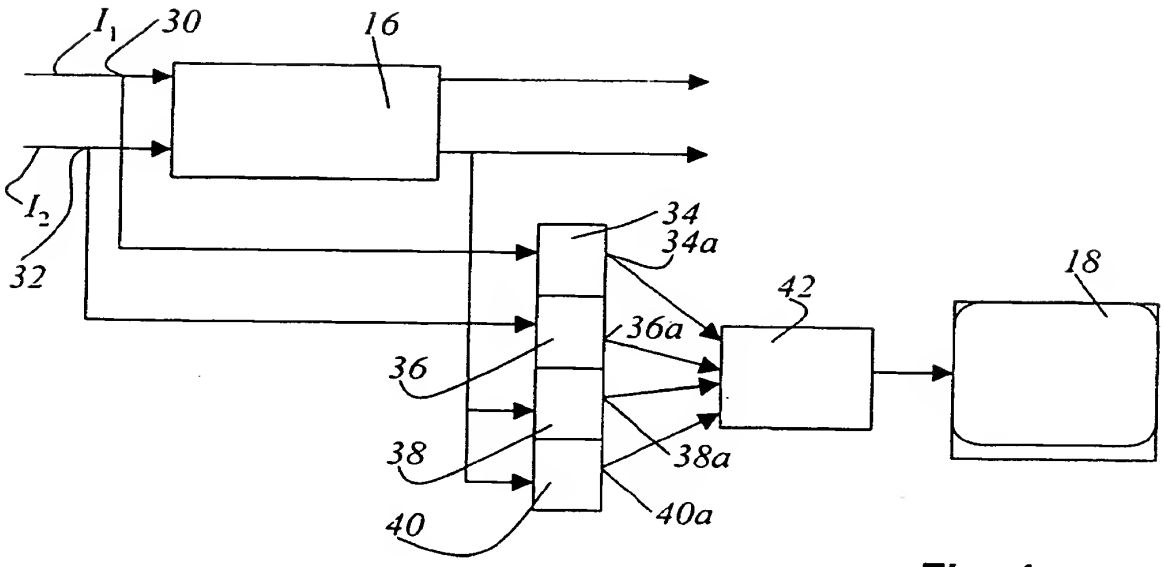
I_1
30
16
I_2
32
34
34a
18
42
36a
36
38
38a
40
40a

Fig. 4

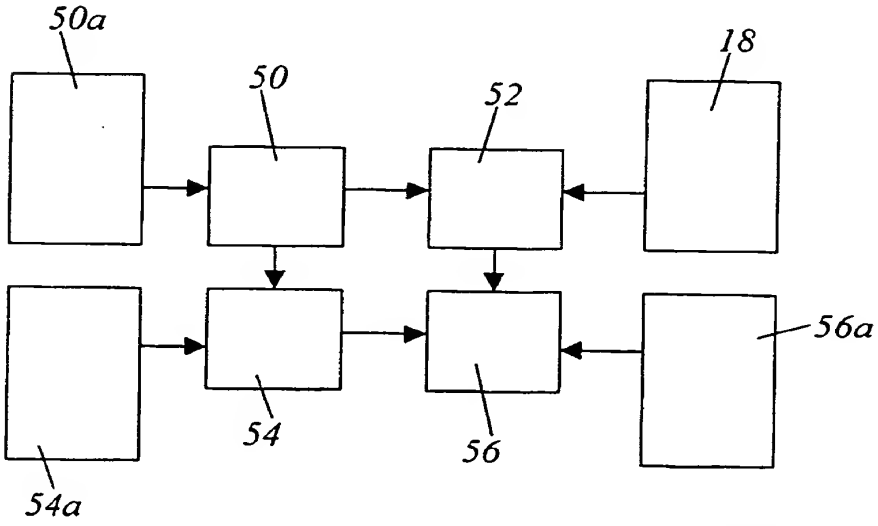
50a
50
52
18
56a
54
56
54a

Fig. 5

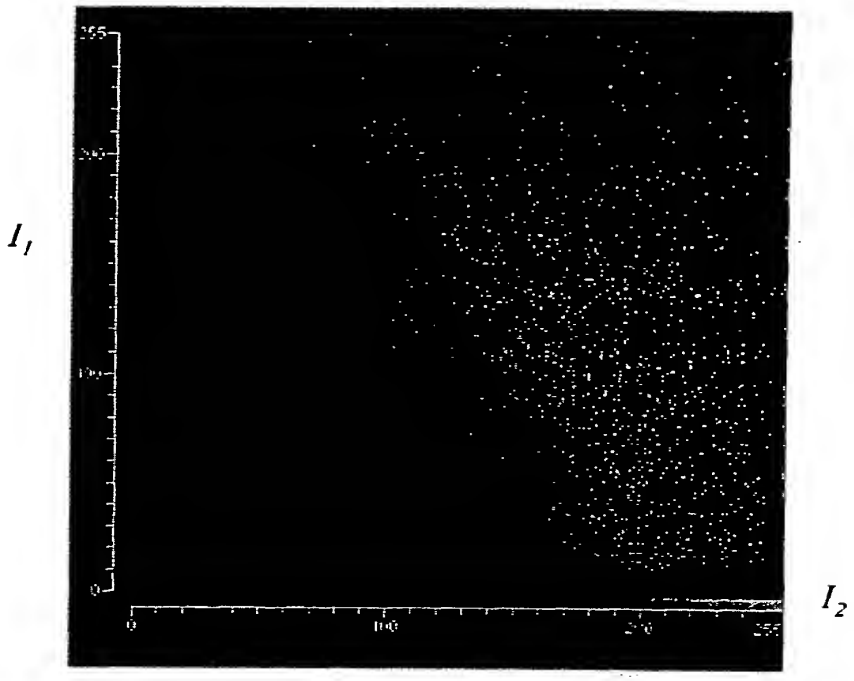

Fig. 7

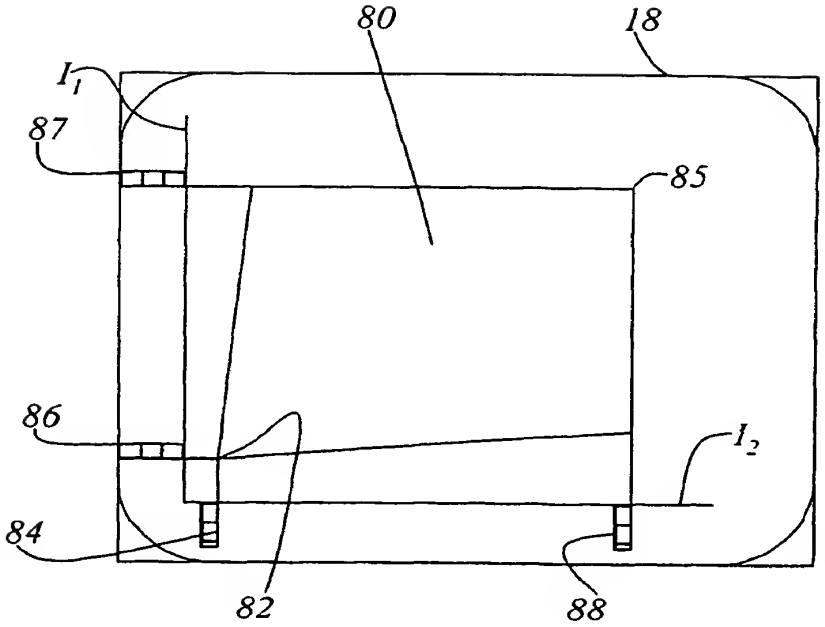

Fig. 8

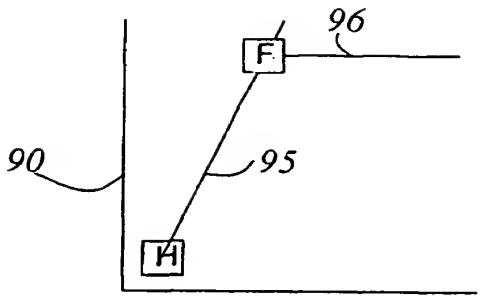

Fig. 9e

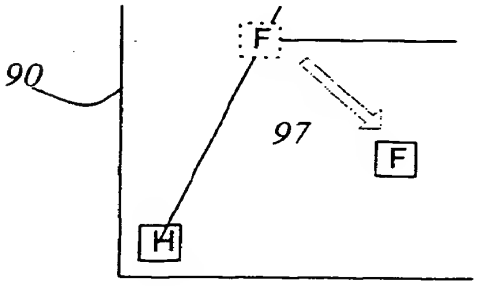

Fig. 9f

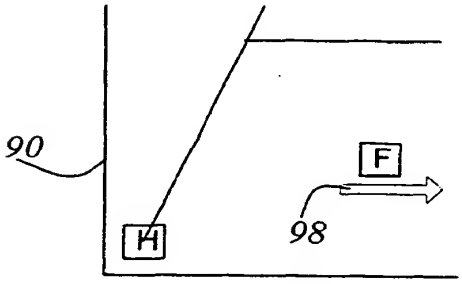

Fig. 9g

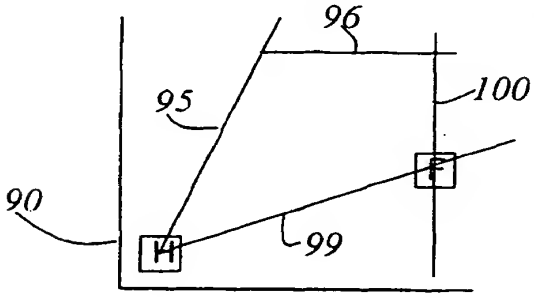

Fig. 9h

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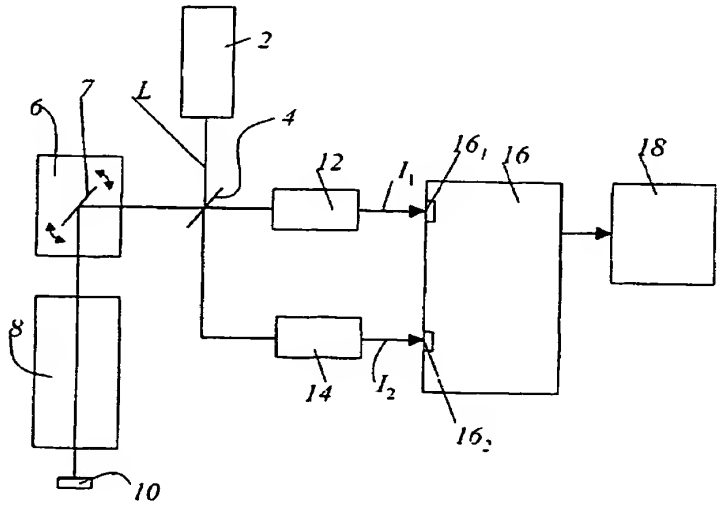

Fig. 1:

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 12 4682

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18-10-2002

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